Vitamin D3, the Skin Immune System and Cutaneous Carcinogenesis

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Abstract. UVB-induced DNA damage and suppression of the skin immune system (SIS) are major aetiological agents in the development of skin cancer. UVB irradiation of the skin also causes the production of vitamin D3. Evidence suggests that local conversion vitamin D3 to its biologically active form may be important in protecting against UVB-induced immunosuppression of the SIS. The aim of this study was to determine if dietary vitamin D3 protects against UVB-induced immunosuppression of the contact hypersensitivity (CHS) response. To assess this an *in vivo* study of UVB-induced suppression of the CHS response in vitamin D3 replete and deficient BALB/c and C57BL/6 mice was undertaken. The level of UVB-induced immunosuppression was significantly higher in vitamin D3 deficient C57BL/6 male and female mice when compared to their vitamin D3 replete counterparts. Vitamin D3 deficiency did not alter the level of UVB-induced immunosuppression in BALB/c mice. The protection against UVB-induced immunosuppression may relate to differences in levels of UVB-induced DNA damage (thymine dimers) of cells of the skin and UVB-induced production of vitamin D3.

Vitamin D3 and Skin Cancer Development

Skin cancer is the most common cancer in the world. The majority of skin cancers are caused by exposure of the skin to ultraviolet B radiation (UVB). UVB irradiation causes DNA damage to cells of the skin and suppression of the SIS. However, UVB irradiation also causes the production of vitamin D3 by keratinocytes that also undergoes sequential enzymatic hydroxylation to become the biologically active form, 1α,25 dihydroxyvitamin D3 (1α,25$(OH)_2$D3) within the skin. Recently an increase in the levels of 1α,25$(OH)_2$D3 in mouse skin following UVB-irradiation has been demonstrated (Biggs *et al.* 2010).

Topically applied 1α,25$(OH)_2$D3 to skin can suppress the SIS but can also protect skin against UVB-induced DNA damage (thymine dimers) and UVB-induced immunosuppression (reviewed in Kuritzky *et al.* 2008).The protection against UVB-induced immunosuppression may contribute to a reduced risk of skin cancer development. The focus of this study was to determine whether dietary vitamin D3 similarly influenced the skin's response to UVB irradiation.

Materials and Methods

An *in vivo* study of UVB-induced suppression of the contact hypersensitivity (CHS) response in vitamin D3 replete and deficient BALB/c and C57BL/6 mice was undertaken. Mice were protected from UVB exposure and commenced on a vitamin D3 deficient diet at three weeks of age. Breeding pairs of vitamin D3 replete and vitamin D3 deficient mice were formed and the off-spring of these mice were used in experiments. Mice were irradiated with a bank of six FS40 sunlamps, in perspex cages, covered with clear polyvinylchloride plastic to exclude wavelengths less than 290nm. The dorsal skin was shaved prior to UVB irradiation and the ears protected by sunscreen (5% 2-ethylhexyl-p-methoxycinnimate). To suppress the SIS mice were exposed to UVB irradiation ((1,1,1,2,2,2 kJ/m^2) and (1,1,1,2,2 kJ/m^2)) in BALB/c and C57BL/6 mice respectively) on consecutive days and compared to similarly treated but sham irradiated mice. Lateral tail vein blood samples were taken for quantification of 25(OH)D3 prior to and 48 hours following the last UVB irradiation. Mice were sensitised with the contact sensitiser oxazolone through UVB or sham irradiated skin, 72 hours following the last UVB irradiation. Ears were challenged 7 days post sensitisation, ear swelling measured 24 hours later, and the percent suppression of the CHS response calculated by comparing the CHS response of sham irradiated mice to UVB irradiated mice. To assess UVB induced DNA damage the dorsal skin from mice was taken following UVB irradiation and the epidermal keratinocytes analysed for the presence of thymine dimers (TD) using immunohistochemistry.

Results

The level of UVB-induced immunosuppression was significantly higher in vitamin D3 deficient C57BL/6 male and female mice when compared to their vitamin D3 replete counterparts. In contrast, vitamin D3 replete and deficient BALB/c mice showed similar levels of UVB-induced immunosuppression. Thus, dietary vitamin D3 protects against UVB-induced immunosuppression of the CHS response in C57BL/6 but not BALB/c mice.

UVB-induced DNA damage is required for UVB-induced suppression of the SIS in mice (Applegate *et al.* 1989). Therefore, the inability of vitamin D3 to protect against UVB-induced immunosuppression in BALB/c mice compared to C57BL/6 mice may reflect differences in the capacity of vitamin D3 to reduce UVB-induced DNA damage and promote DNA repair. Vitamin D3 did not lower the initial UVB-induced mean percentage of TD positive keratinocyte nuclei in male or female C57BL/6 mice. However, vitamin D3 did lower the intensity of TD staining, a factor that reflects the lower numbers of TD within the nucleus, in both male and female C57BL/6 mice. Conversely, vitamin D3 did not significantly influence either the percentage of TD positive nuclei or level of TD staining in BALB/c male or female mice.

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Promotion of UVB-induced DNA repair is also associated with reduced UVB-induced immunosuppression (Applegate *et al.* 1989). The rate of disappearance of TD positive nuclei was estimated as an indicator of whether vitamin D3 influenced DNA repair. Vitamin D3 significantly reduced the percentage of keratinocyte nuclei containing TD in C57BL/6 male mice, and a trend for reduction was identified in C57BL/6 female mice, 48 hours after UVB irradiation. Conversely, vitamin D3 did not alter the reduction in TD nuclei over a 72 hour period in BALB/c mice,

UVB irradiation caused a significant elevation in serum 25(OH)D3 levels in both BALB/c and C57BL/6 vitamin D3 deficient mice. Despite receiving one less UVB irradiation the C57BL/6 mice had similar 25(OH)D3 levels as their BALB/c counterparts. The elevation in 25(OH)D3 was significantly higher in female mice, than male mice, for both strains.

Discussion

The availability of 25(OH)D3 protected against UVB-induced immunosuppression of the CHS response in the C57BL/6 but not BALB/c mice. Vitamin D3 did not lower the initial UVB-induced mean percentage of TD positive keratinocyte nuclei in male or female C57BL/6 mice. However, vitamin D3 did lower the intensity of TD staining, a factor that reflects the lower numbers of TD within the nucleus, in both male and female C57BL/6 mice. This suggests that vitamin D3 subtly reduces the level of TD in C57BL/6 mice and thus may contribute to protection against UVB-induced immunosuppression in these mice. Conversely, vitamin D3 did not significantly influence either the percentage of TD positive nuclei or level of TD staining in BALB/c male or female mice and did not protect against UVB-induced immunosuppression. These findings suggest that vitamin D3 photoprotection against UVB-induced DNA damage in C57BL/6 mice may account for the reduced UVB-induced immunosuppression in vitamin D3 replete C57BL/6 mice. Consequently, vitamin D3 protection against UVB-induced DNA damage may be dependent on the genetic background of the mice.

This suggests that vitamin D3 also promotes the repair of TD, which may lead to reduced UVB-induced immunosuppression in C57BL/6 mice. Conversely, vitamin D3 did not alter the reduction in TD nuclei over a 72 hour period in BALB/c mice, or reduce UVB-induced immunosuppression in BALB/c mice. These results indicate that photoprotection by vitamin D3 in C57BL/6 mice is related to reduced UVB-induced DNA damage and potentially enhanced repair. These results also suggest that genetic differences influencing the susceptibility to UVB-induced immunosuppression may relate to DNA repair mechanisms. A compromise in the repair of UVB-induced TDs in neonatal $VDR^{-/-}$ mice has also been demonstrated (Ellison *et al.* 2008).

Kuritzky *et al.* (2008) suggested that $1\alpha,25(OH)_2D3$ produced from one UVB exposure is unlikely to exert photoprotective effects in the skin, as the production of $1\alpha,25(OH)_2D3$ in the UVB irradiated epidermis takes several hours, but photoprotection could occur after subsequent UVB exposures. Therefore, as vitamin D3 contributed to a potential improvement in DNA repair in C57BL/6 mice, the repeated UVB irradiation used to induce local immunosuppression may have resulted in cumulatively less DNA damage in C57BL/6 mice. Less DNA damage would also contribute to the protection against UVB-induced immunosuppression conferred by vitamin D3 in C57BL/6 mice.

The levels of $1\alpha,25(OH)_2D3$ achieved in the skin following UVB irradiation may also influence the rate of DNA repair. Skin production of vitamin D3 in BALB/c and C57BL/6 mice may be inferred from the rise in the serum 25(OH)D3 levels following UVB irradiation. C57BL/6 mice had similar rises in 25(OH)D3 as BALB/c mice after UVB-irradiation, even though they received one less $2kJ/m^2$ dose of UVB-irradiation. This could indicate that C57BL/6 mice produce more vitamin D3 and $1\alpha,25(OH)_2D3$ locally in the skin. If the rise in serum 25(OH)D3 levels in C57BL/6 mice do reflect higher levels of UVB-induced $1\alpha,25(OH)_2D3$ within the skin, compared to BALB/c mice, this may contribute to the increased sensitivity of C57BL/6 mice to UVB-induced immunosuppression when compared to BALB/c mice. However, greater local production of $1\alpha,25(OH)_2D3$ may contribute to the enhanced DNA repair in C57BL/6 mice.

The extent of UVB-induced immunosuppression will depend on the balance between $1\alpha,25(OH)_2D3$ mediated photoprotection against DNA damage and the promotion of DNA repair (and therefore a reduction in UVB-induced immunosuppression) and the inherent ability of locally produced $1\alpha,25(OH)_2D3$ to suppress the SIS.

References

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